Table S1. Comparison of web-based oligonucleotide design tools. The comparison between different oligonucleotide design/check tools shows that most of the tools did not involve specificity and cross dimer checks. Therefore, these tools also don't involve the possibility for multiplexing. Except for oli2go, which includes all listed design principles, MFEprimer involves most of the processing steps. However, it only provides limited access to background data for specificity checks and less comprehensive cross dimerization calculations.

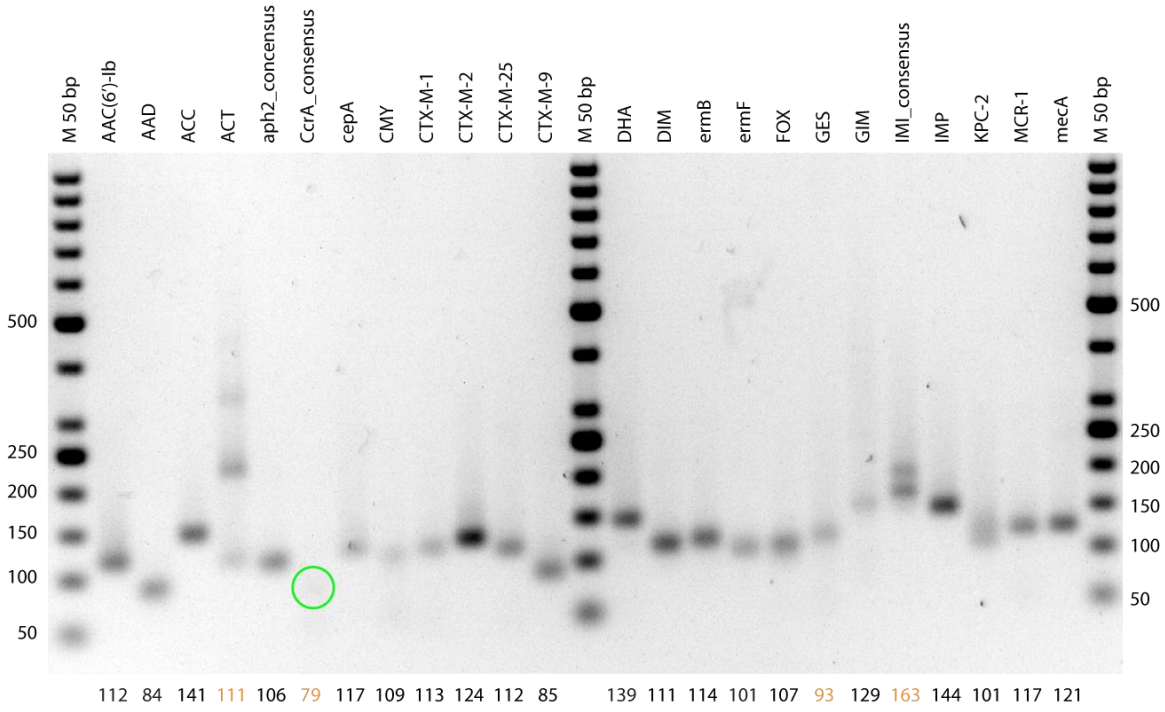
Tool	Primer Design	Probe Design	Hairpin Check	Batch design*	Probe Specificity Check	Primer Specificity Check	Cross Dimer Check	Multiplexing
Oli2go	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
MSP-HTPrimer	Yes	Yes	Yes	Yes	No	No	No	No
PrecisePrimer	Yes	No	No	Yes	No	No	No	No
Primer3	Yes	Yes	Yes	No	No	No	No	No
MFEprimer	No	No	Yes	Yes	Yes (limited)	Yes	Yes	Yes (limited)
Primer-BLAST	Yes	No	No	No	No	Yes	No	No

* Batch design means the application of the software on more than one input sequences in one run. However, this term does not cover multiplexing, as design steps such as specificity and secondary structure checks need to be performed for multiplex applications.

Table S2. Comparison between cross dimer checks using oli2go and MFEprimer. Primer sequences of 6 genes (ceoA: fwd – TTCAAGGACGGCGCG, rev – TATAGCCGAGRTTGATGCG; cphA4: fwd – GCGAGCTGCACAAGCT, rev – CCTTGCGGGTAAAGGC; cfiA7: fwd – TTATCCTTATCTCCATGCTT, rev – TTCGGCGAGGGATACATAAGT, OXY2-5: fwd – GTGCAGCACCAGTAAAG, rev – CGTTAATCTCCAGCCTTT; ykkC: fwd – TTAACATGGAGCGGCACT, rev – TTATGCCTCGCCTCCT; ykkD: fwd – ATGCTGCACTGGATCAGTTTA, rev - GCAAAACCAACAATGATCAACAG) were used to compare cross dimerization check results of MFEprimer and oli2go. Parameters for both tools were chosen as follows: concentration of monovalent cations 10 mM, concentration of divalent cations 22 mM, concentration of dNTPs 1.75 mM, primer concentration 50 nM, deltaG threshold -10.000 cal/mol and a T_m threshold of 40 °C. First, MFEprimer does not involve the possibility to define thresholds for deltaG and the T_m of secondary structures. Therefore, resulting secondary structures must be examined manually. If we apply the same threshold for MFEprimer as used for oli2go, there would be no cross dimerization resulting from MFEprimer. However, oli2go would detect 2 primer dimers using the same parameters as with MFEprimer. This difference in results of both software packages arises from the fact, that MFEprimer involves less thermodynamical parameters and methods for finding the most stable structure. Furthermore, oli2go compares the melting temperature and the delta G value of the secondary structure. If only one of these parameters exceeds the threshold, the cross dimer check fails. However, MFEprimer only returns delta G values of the secondary structures. These characteristics lead to a less stringent evaluation of secondary structures.

Tool	Primer 1	Primer 2	DeltaG	T_m	Dimerisation
Oli2go	cfiA7 rev primer	ykkC ref primer	-11324.5	37.43	True
	OXY2-5 fwd primer	ykkD fwd primer	-10246.7	32.51	True
MFEprimer	cphA4 rev primer	OXY2-5 rev primer	-7600.0	-	-

Figure S1. Agarose gel image of experimental primer evaluation experiments. Primers were evaluated in single-plex PCRs to analyze the functionality and specificity of the respective primer pairs. The PCR was conducted using the DNA-free Mastermix 16S Basic PCR kit (Molzym, Germany) according to the manufacturer's instructions comprising 200 nM of each primer, 1 µl of target DNA and 0.32 µl of the Moltaq 16S polymerase in a total volume of 10 µl. The thermal cycling was performed as follows: 94°C for 5 min; 40 cycles of 94°C for 30 s, 46°C for 30 s, 72°C for 30 s; and a final elongation cycle at 72°C for 7 min. The highlighted numbers indicate deviations from the expected amplification product size or low amplification efficiencies. In such cases, the primers have to be redesigned if experimental mistakes can be excluded.



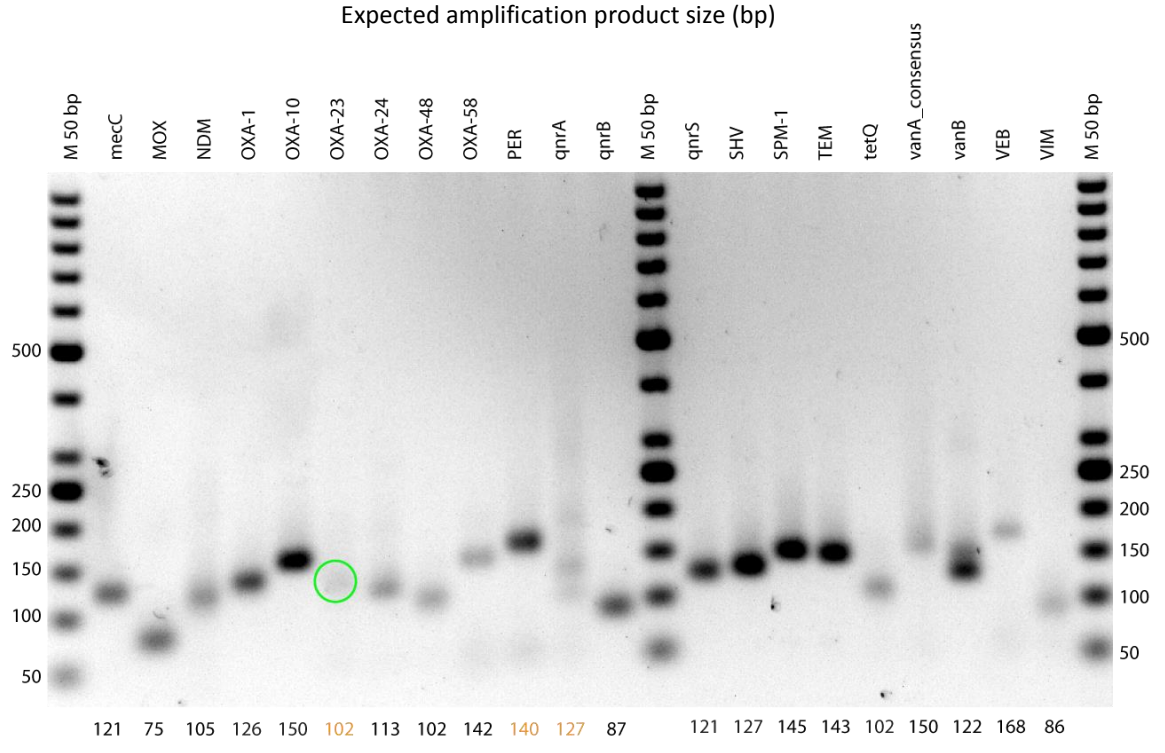
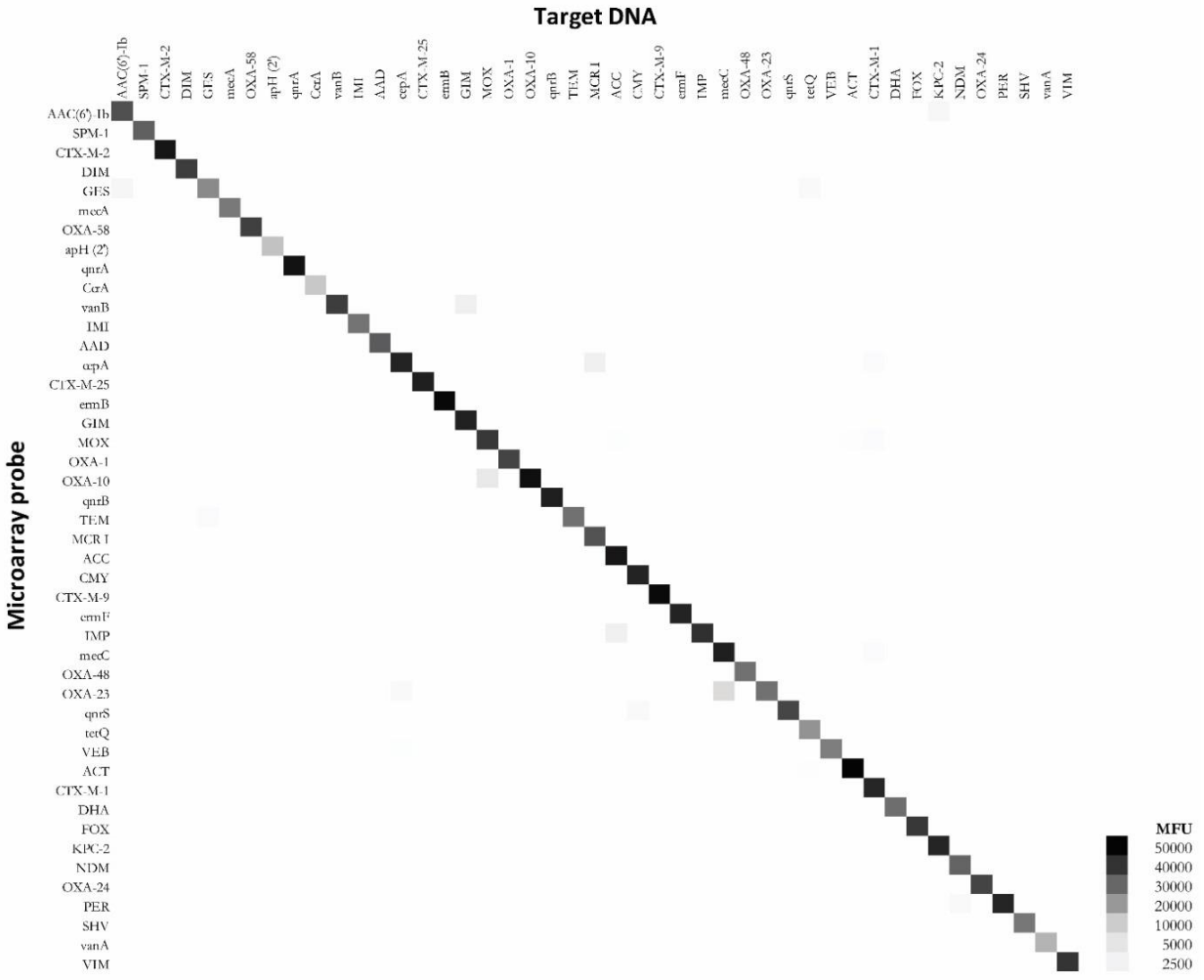


Figure S2. Heatmap illustrating microarray fluorescence signals (MFU) from 45 experiments using antibiotic resistance genes as target sequence. To achieve a high specificity in the solid-support based detection reaction, the LNC3-probe detection technology was used as previously described by Barišić et al. (1). The experimental procedure was slightly modified and biotin-labelled detection oligonucleotides instead of fluorescently labelled were used. The detection reaction was as follows; custom, in-house functionalized glass slides were spotted with LNC3-based microarray probes using an OmniGrid contact arrayer (GeneMachines, San Carlos, CA, USA) as previously described. In a subsequent ligation reaction, detection oligonucleotides were in the presence of a DNA target covalently attached to the LNC3-probe. The ligation was realized in four array gasket hybridization chambers with a capacity of 100 µl each (Agilent, Santa Clara, CA, USA). Onto the hybridization chamber, the ligation solution was pipetted, comprising ampligase (5 U/reaction, Epicentre, Madison, WI, USA), ampligase buffer (20 mM Tris-HCl, 25 mM KCl, 10 mM MgCl2, 0.5 mM nicotinamide adenine dinucleotide (NAD), 0.01 % Triton X-100, pH=8.3), 5 µg bovine serum albumin (BSA, New England BioLabs), the enzymatically modified detection oligonucleotides at a final concentration of 30 nM each, and 0.1 µM of target DNA. The ligation took place in a hybridization oven at 55 °C for 1 h. Afterwards, the slides were washed for 5 min with 2x SSC including 0.1 % SDS. Subsequently, the slides were washed with 0.2x SSC for 2 min and finally washed twice with ddH2O for 1 min. To get rid of unspecific hybridization products and consequently false positive signals, the slides were washed with ddH2O for 10 min at 70 °C. Due to the biotin labelling, it was possible to use a Streptavidin-Alexa-647 conjugate (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA), diluted 1:1000 in 1x PBS, including 0.1 % Tween, to obtain fluorescence signals. The slides were centrifuged to dry and scanned with a Tecan PowerScanner (Männedorf, Switzerland). The data were analyzed with GenePix Pro 6.0 (Molecular Devices, Sunnyvale, CA, USA) and Excel 2010 (Microsoft, Redmond, WA, USA).



REFERENCES

1. Barišić, I., Kamleithner, V., Schönthaler, S. and Wiesinger-Mayr, H. (2014) Fast and highly specific DNA-based multiplex detection on a solid support, *Applied microbiology and biotechnology*.